Supporting Information

Porter *et al*. 10.1073/pnas.0808010105

SI Text

Effect of Variation of [$CheA_3$] on the Dephosphorylation Rate of $CheY_6$-P. The dephosphorylation rate of $CheY_6$-P was measured in the presence of varying amounts of $CheA_3$ (Fig. S1). As the concentration of $CheA_3$ was increased, the rate of $CheY_6$-P dephosphorylation increased, with no evidence of saturation over the concentration range tested. The limited solubility of $CheA_3$ coupled with the high concentrations of CheY and phosphodonor needed for this assay, imposed the upper limit of the $CheA_3$ concentration range tested in these assays of 2.5 μM. The shape of the curve is suggestive of sigmoidal kinetics with respect to $CheA_3$, possibly indicating either positive cooperativity or some need for $CheA_3$ to oligomerize to function as a phosphatase. Either way, these data suggest that higher concentrations of $CheA_3$ might be able to stimulate $CheY_6$-P dephosphorylation by more than the factor of 3 seen for 2.5 μM $CheA_3$.

$CheA_3$ Is Not a Homologue of the CheC Family of Phosphatases. Sequence analysis of the 794-amino acid region between the P1 and P5 domain of $CheA_3$ revealed a partial match (**D**-X_4-**E**-X_2-**N**-X_{20}-**P**) to the consensus sequence of the CheC family of CheY-P phosphatases (**D/S**-X_3-**E**-X_2-**N**-$X_{21/22}$-**P**) (1, 2). The E and N residues within this consensus sequence have been shown to be essential for phosphatase activity in a number of CheC family members (2, 3). To determine whether $CheA_3$ uses a similar phosphatase mechanism, the corresponding residues in $CheA_3$ (E585 and N588) were both changed to serine and the resulting protein assayed for $CheY_6$-P phosphatase activity. The $CheA_3$(E585S,N588S) protein displayed wild-type levels of $CheY_6$-P phosphatase activity (Fig. 3) indicating that $CheA_3$ is not a member of the CheC family of phosphatases.

$CheA_3$ Does Not Show Phosphatase Activity Toward $CheA_2$-P or $CheA_3$P1-P. Experiments where $CheA_3$ was incubated alone with the phosphodonors, $CheA_2$-P and $CheA_3$P1-P, in the absence of added RRs, detected no change in the concentration of phosphodonor over time (Fig. S2), showing that $CheA_3$ does not possess histidyl-phosphate phosphatase activity toward either of the phosphodonors used in this study.

1. Szurmant H, Bunn MW, Cannistraro VJ, Ordal GW (2003) Bacillus subtilis hydrolyzes CheY-P at the location of its action: The flagellar switch. *J Biol Chem* 278:48611–48616.
2. Muff TJ, Ordal GW (2007) The CheC phosphatase regulates chemotactic adaptation through CheD. *J Biol Chem* 282:34120–34128.
3. Park SY, *et al.* (2004) Structure and function of an unusual family of protein phosphatases: The bacterial chemotaxis proteins CheC and CheX. *Mol Cell* 16:563–574.
4. Penfold RJ, Pemberton JM (1992) An improved suicide vector for construction of chromosomal insertion mutations in bacteria. *Gene* 118:145–146.
5. Sockett RE, Foster JCA, Armitage JP (1990) Molecular biology of the *Rhodobacter sphaeroides* flagellum. *FEMS Symp* 53:473–479.
6. Porter SL, Armitage JP (2004) Chemotaxis in *Rhodobacter sphaeroides* requires an atypical histidine protein kinase. *J Biol Chem* 279:54573–54580.
7. Porter SL, *et al.* (2006) The CheYs of *Rhodobacter sphaeroides*. *J Biol Chem* 281:32694–32704.
8. Porter SL, Warren AV, Martin AC, Armitage JP (2002) The third chemotaxis locus of *Rhodobacter sphaeroides* is essential for chemotaxis. *Mol Microbiol* 46:1081–1094.
9. Wadhams GH, Warren AV, Martin AC, Armitage JP (2003) Targeting of two signal transduction pathways to different regions of the bacterial cell. *Mol Microbiol* 50:763–770.
10. Shah DS, *et al.* (2000) Identification of a fourth *cheY* gene in *Rhodobacter sphaeroides* and interspecies interaction within the bacterial chemotaxis signal transduction pathway. *Mol Microbiol* 35:101–112.
11. Porter SL, Armitage JP (2002) Phosphotransfer in *Rhodobacter sphaeroides* chemotaxis. *J Mol Biol* 324:35–45.
12. Martin AC, *et al.* (2001) CheR- and CheB-dependent chemosensory adaptation system of *Rhodobacter sphaeroides*. *J Bacteriol* 183:7135–7144.
13. Schäfer A, *et al.* (1994) Small mobilizable multipurpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19 - Selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene* 145:69–73.

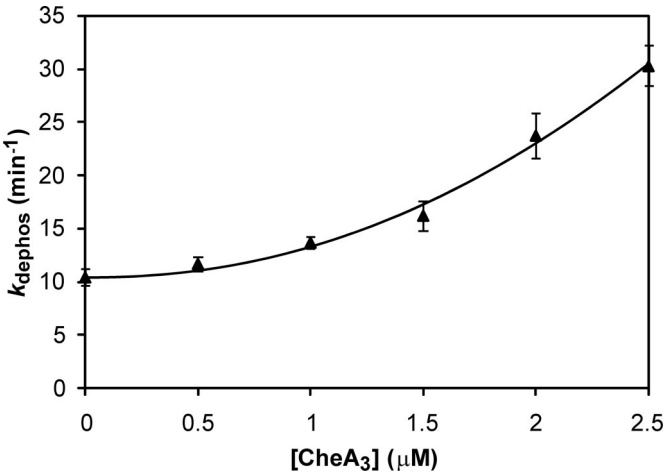

Fig. S1. The effect of varying the concentration of $CheA_3$ on the rate of $CheY_6$-P dephosphorylation. Reactions contained 30 μM $CheA_3$P1-P, 400 μM $CheY_6$, and the concentration of $CheA_3$ was varied between 0 μM and 2.5 μM. Error bars show standard error of the mean obtained from six replicates.

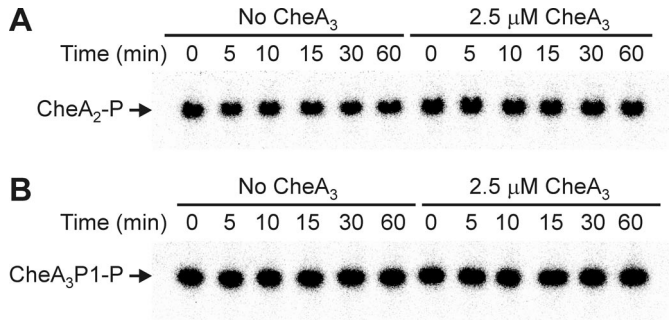

Fig. S2. Phosphorimages of SDS/PAGE gels showing the effect of $CheA_3$ on levels of $CheA_2$-P and $CheA_3$P1-P. (*A*) 2 μM $CheA_2$-P was incubated in the absence (left half of gel) and presence of 2.5 μM $CheA_3$ (right half of gel). (*B*) 2 μM $CheA_3$P1-P was incubated in the absence (left half of gel) and presence of 2.5 μM $CheA_3$ (right half of gel). Ten-μl reaction samples were taken at the time points indicated and quenched in 20 μl of 1.5$\times$ SDS/EDTA loading dye. The quenched samples were analyzed by SDS/PAGE and detected by phosphorimaging.

Table S1. Plasmids and bacterial strains used in this study

Strain/plasmid	Description	Source/reference
E. coli strains		
M15pREP4	Expression host containing pREP4; kanamycin resistant	Qiagen
S17–1λ*pir*	Strain capable of mobilizing the suicide vector pK18*mobsacB* into *R. sphaeroides*; streptomycin resistant	(4)
XL1 Blue	General cloning strain and expression host. *lacI^q; tetracycline resistant*	Stratagene
R. sphaeroides strains		
WS8N	Spontaneous nalidixic acid resistant mutant of wild type WS8	(5)
JPA1210	WS8N containing *cheA$_3$(H51Q) in place of the wild-type cheA$_3$ in the chromosome. Non-chemotactic*	(6)
JPA1213	WS8N containing *cheY$_6$(D56N) in place of wild-type cheY$_6$ in the chromosome. Non-motile*	(7)
JPA1314	D*cheA$_3$ derivative of WS8N. Non-chemotactic*	(8)
JPA1425	WS8N containing a *yfp–cheA$_3$ fusion in place of the wild-type cheA$_3$ in the chromosome*	(9)
JPA1739	WS8N containing *cheA$_3$(D155–948) in place of the wild-type cheA$_3$ in the chromosome*	This study
JPA1741	WS8N containing a *yfp–cheA$_3$(D155–948) fusion in place of the wild-type cheA$_3$ in the chromosome*	This study
Plasmids		
pGEX6P-1	IPTG inducible expression vector. Introduces a GST tag at the N terminus of the expressed protein. Confers ampicillin resistance	GE Life Sciences
pGEXA4	CheA$_4$ expression plasmid. pGEX6P-1 derivative	This study
pREP4	Plasmid containing the *lacI^q gene. Compatible with pQE30 and pQE60. Confers kanamycin resistance*	Qiagen
pQE30	IPTG inducible expression vector. Introduces RGS(H)$_6$ at the N terminus of the expressed protein. Confers ampicillin resistance	Qiagen
pQE60	IPTG inducible expression vector. Introduces RGS(H)$_6$ at the C terminus of the expressed protein. Confers ampicillin resistance	Qiagen
pQEA2	CheA$_2$ expression plasmid. pQE30 derivative	(10)
pQE60A3	CheA$_3$ expression plasmid. pQE60 derivative	(6)
pQE60A3P1	CheA$_3$P1 expression plasmid. pQE60 derivative	This study
pQE60A3(H51Q)	CheA$_3$(H51Q) expression plasmid. pQE60 derivative	(6)
pQE60A3(Δ155–948)	CheA$_3$(Δ155–948) expression plasmid. pQE60 derivative	This study
pQE60A3(Δ2–154)	CheA$_3$(Δ2–154) expression plasmid. pQE60 derivative	This study
pQE60A3(Δ155–349)	CheA$_3$(Δ155–349) expression plasmid. pQE60 derivative	This study
pQE60A3(Δ349–549)	CheA$_3$(Δ349–549) expression plasmid. pQE60 derivative	This study
pQE60A3(Δ549–749)	CheA$_3$(Δ549–749) expression plasmid. pQE60 derivative	This study
pQE60A3(Δ749–948)	CheA$_3$(Δ749–948) expression plasmid. pQE60 derivative	This study
pQE60A3(Δ950–1095)	CheA$_3$(Δ950–1095) expression plasmid. pQE60 derivative	This study
pQE60A3(Δ155–549)	CheA$_3$(Δ155–549) expression plasmid. pQE60 derivative	This study
pQE60A3(Δ155–749)	CheA$_3$(Δ155–749) expression plasmid. pQE60 derivative	This study
pQE60A3(Δ549–948)	CheA$_3$(Δ549–948) expression plasmid. pQE60 derivative	This study
pQE60A3(Δ349–948)	CheA$_3$(Δ349–948) expression plasmid. pQE60 derivative	This study
pQE60A3(E585S,N588S)	CheA$_3$(E585S,N588S) expression plasmid. pQE60 derivative	This study
pQEA4	CheA$_4$ expression plasmid. pQE30 derivative	(8)
pQEY1	CheY$_1$ expression plasmid. pQE30 derivative	(10)
pQEY2	CheY$_2$ expression plasmid. pQE30 derivative	(10)
pQEY3	CheY$_3$ expression plasmid. pQE30 derivative	(10)
pQEY4	CheY$_4$ expression plasmid. pQE30 derivative	(10)
pQEY5	CheY$_5$ expression plasmid. pQE30 derivative	(11)
pQEY6	CheY$_6$ expression plasmid. pQE30 derivative	(8)
pQEB1	CheB$_1$ expression plasmid. pQE30 derivative	(12)
pQEB2	CheB$_2$ expression plasmid. pQE30 derivative	(8)
pK18*mobsacB*	Allelic exchange suicide vector mobilized by *E. coli* S17–1λ*pir*. Confers kanamycin resistance and sucrose sensitivity	(13)
pK18A3(Δ155–948)	Construct for replacing *cheA$_3$* -with *cheA$_{3(Δ155–948)}$* in *R. sphaeroides genome. pK18mobsacB* derivative	This study
pK18A3(E585S,N588S)	Construct for replacing *cheA$_3$* -with *cheA$_{3(E585S,N588S)}$* in *R. sphaeroides genome. pK18mobsacB* derivative	This study